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Addendum

The role of phytosterols in plant adaptation to temperature

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Membranes of composition approaching those found in "rafts" of plants, fungi and mammals were investigated by means of solid-state ^{2}H-NMR, using deuterated dipalmitoyl-phosphatidylcholine (^{2}H-DPPC) as a reporter. The dynamics of such membranes was determined through measuring of membrane ordering or disordering properties. The presence of the liquid-ordered, *lo*, phase, as an indicator of rigid sterol-sphingolipid domains, was detected in all cases. Of great interest, the dynamics of mixtures mimicking rafts in plants showed the lesser temperature sensitivity to thermal shocks. The presence of an additional ethyl group branched on the alkyl chain of major plant sterols (sitosterol and stigmasterol) is proposed as reinforcing the membrane cohesion. The fine tuning of the sterol structure thus appears to be the evolution response for plant adaptation to large temperature variations.

It is widely recognized that lipids play multiple roles that either individually or collectively influence cell processes. Glycerolipids and sphingolipids through charge and structure are involved in DNA replication, protein translocation, cell recognition, signalling pathways, energetic, signal transduction, and cell trafficking. Together with diacylglycerols their collective properties modulate lipid polymorphism, through phase transitions (lamellar, hexagonal, cubic, micelles), which are involved in enzyme conformational changes, cell division, cell fusion, and apoptosis.[1]

Sterols, the third lipid class, also regulate biological processes and sustain the domain structure of cell membranes where they are considered as membrane reinforcers.[2,3] While cholesterol (CHO) is the major sterol of vertebrates, ergosterol plays a key role in fungi. Plants usually possess more complex sterol compositions. Stigmasterol (STI) and sitosterol (SIT), two 24-ethyl sterols, are major constituents of the sterol profiles of plant species. They are involved in the embryonic growth of plants.[4,5] Sterols are critical for the formation of liquid-ordered (*lo*) lipid domains (lipid rafts) that are supposed to play an important role in fundamental biological processes like signal transduction, cellular sorting, cytoskeleton reorganization and infectious diseases.[6,7] In plants, specialized lipid domains are involved in the polarized growth of pollen tube and root hair[8] and the asymmetric growth of plant cells is in general due to the asymmetric distribution of membrane components.

We recently documented the effect of sitosterol and stigmasterol, two major plant sterols, on the structure and dynamics of membranes whose composition is representative of domains (rafts) in plants.[9] Liposomes of phytosterols associated with glucosylcerebroside (GC) and with deuterium-labelled dipalmitoylphosphatidylcholine (^{2}H-DPPC) were analysed with deuterium solid state nuclear magnetic resonance (^{2}H-NMR). For comparison, membrane systems representative of raft composition in fungi and mammals were also investigated. ^{2}H-NMR is known to be the best non-invasive technique to analyse membrane dynamics[10] because it is non-destructive and because replacement of DPPC protons with their deuterium isotope brings very little membrane perturbation.[11,12] Acyl chain deuteration affords analysis of both structure and dynamics of the hydrophobic membrane interior. Spectra such as that shown in Figure 1 insert, allow detection of the *lo* phase, characteristic of a membrane state half-way between solid-ordered (*so*) and liquid-disordered (*ld*) states. The *so* state, also called "gel", is found at low temperatures (below 35°C), when membranes are essentially composed of sphingomyelins (SM)[13] or GC (Fig. 1). This membrane state allows little biological function because in forbids membrane trafficking due to its very rigid state (order parameter close to 1). In turn, the *ld* or "fluid" state is found at high temperatures, in the absence of SM, GC and sterols (low order parameter). At the opposite such a high membrane dynamics may lead to excessive membrane passages. Following with ^{2}H-NMR the temperature behaviour of membrane systems containing GC and plant sterols, we found that the *so-ld*, order-disorder, transition was totally abolished: SIT and STI fluidized the *so* state and ordered the *ld* state to produce the *lo* state where membrane fluctuations vary smoothly with temperature (Fig. 1). This effect was already documented with CHO in mammals[14-16] but on a much narrower temperature range. The case of the fungus system was found in between that of plants and mammals.

Summarizing, it appears that plant membranes of "raft" composition are less sensitive to temperature variations than those of animals. This suggests that cell membrane components like sitosterol, stigmasterol and glucosylcere-brosides, which are typical of plants, are produced in order to extend the temperature range in which membrane-associated biological processes can take place. This observation is well in accordance with the fact that plants have to face higher temperature

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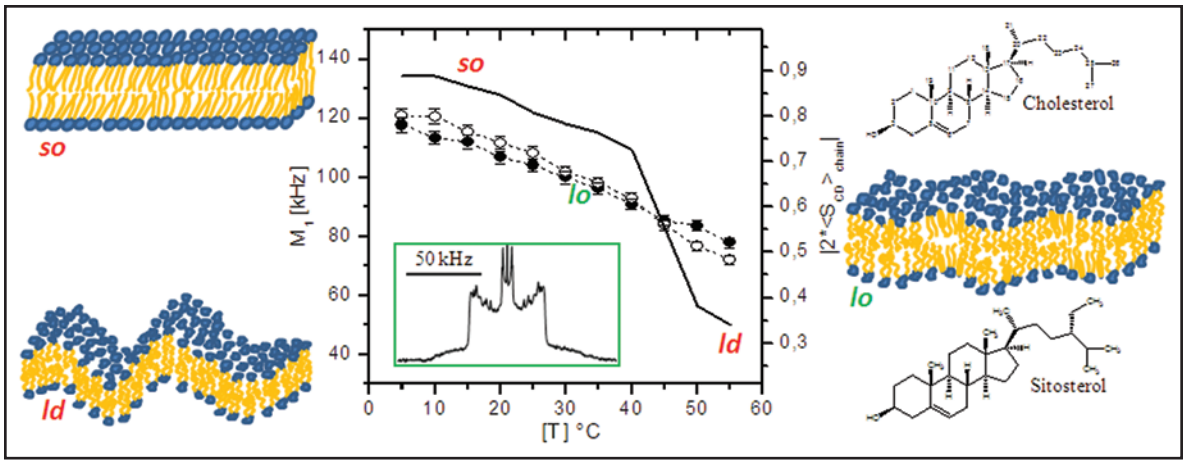

Figure 1. Regulation of temperature-driven membrane dynamics by plant sterols. Central panel: first spectral moment (left y-axis) or order parameter (right y-axis) as a function of temperature; solid line: ^{2}H-DPPC with glucosylcerebroside; open circles: plus stigmasterol; filled circles: plus sitosterol. Insert: ^{2}H-NMR spectrum typical of a liquid-ordered, *lo*, state. Left panel: schematics of solid-ordered, *so* (gel), and liquid-disordered, *ld* (fluid), membrane states. Right panel: schematics of the *lo* (raft) membrane state together with the structures of cholesterol and sitosterol. Adapted from reference 9.

variations than animals, which usually can either regulate their body temperature or change their location in order to avoid extreme heat or coldness.

Compared to cholesterol, the two phytosterols possess additional ethyl groups branched on C-24 (Fig. 1). We proposed that the presence of an additional ethyl group may reinforce the attractive van der Waals interactions leading to more membrane cohesion and therefore less temperature sensitivity. Our results also suggest that domains of smaller size would be promoted in the presence of phytosterols and especially with sitosterol. Such domains may be viewed as dynamic, with sterols laterally exchanging at the microsecond time scale.[14] In plant cells, enzymes transfer alkyl groups to the C-24 of sterols. If we suppose that the relative activities of the different branches of the plant sterol biosynthesis are regulated, the concentrations of major sterols in plants, like sitosterol, stigmasterol, and cholesterol could be controlled.[4,17] This shows the importance of equilibrated sterol concentrations for plant growth and development. Sterols have been historically considered as membrane reinforcers because they bring order to membranes.[2,3]

Our works[9,15,16,18,19] show that they could better be named as "membrane dynamics regulators", by maintaining the membrane in a state of microfluidity suitable for cell function on large temperature scales. It thus appears that a fine tuning of the sterol structure, i.e., the presence of branched ethyl groups in plant sterols increasing membrane cohesion through formation of smaller membrane domains, may be the evolution response for plant adaptation to large temperature variations.

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